Female renal health

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HIGHER FILTRATION FRACTION IN FORMERLY EARLY-ONSET PREECLAMPTIC WOMEN WITHOUT COMORBIDITY

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ABSTRACT

Formerly preeclamptic women have an increased risk for developing end stage renal disease that has been attributed to altered renal hemodynamics and abnormalities in the renin-angiotensin aldosterone system. Whether this is due to preeclampsia itself or to co-morbid conditions is unknown. Renal hemodynamics and responsiveness to angiotensin II during low sodium (7 days 50 mmol Na^+/24h) and high sodium intake (HS; 7 days 200 mmol Na^+/24h) were studied in 18 healthy normotensive formerly early-onset preeclamptic women (fPE-women) and 18 healthy controls (fHP-women), all selected for absence of co-morbidity. At the end of each diet, renal hemodynamics and blood pressure were measured before and during graded angiotensin II infusion. Both HS intake and former preeclampsia increased filtration fraction (FF) without an interaction between the two. FF was highest during HS in fPE-women (0.31 ± 0.12 vs fHP-women: 0.29 ± 0.11, GEE analysis corrected for BMI, p=0.03). Renal response to angiotensin II infusion was not different between the groups. In conclusion, fPE-women have a higher FF compared to fHP-women. Since we observed a mildly elevated FF in fPE-women, in the absence of co-morbidity, it could be that preeclampsia itself exerts long-term effects on renal hemodynamics. On the other hand, mild pre-pregnancy changes in renal function could be present and lead to increased risk for preeclampsia. In experimental studies a pathogenetic role of elevated FF has been shown in the development of hypertension and renal damage. Future studies should evaluate whether our subtle differences in renal hemodynamics lead to renal dysfunction in the long-term in fPE-women.

INTRODUCTION

Complicating up to 8% of pregnancies, preeclampsia (PE) is a major cause of maternal and fetal morbidity and mortality worldwide.[1] PE is characterized by de-novo development of hypertension and proteinuria during the second half of pregnancy. Although it is a pregnancy-specific disease, evidence has mounted that PE has important long-term implications for maternal health, in particular cardiovascular and renal health.[2-4] It is, however, uncertain whether the increased renal and cardiovascular risk in formerly preeclamptic women is explained by PE itself, or by underlying common (pre-pregnant) risk factors and co-morbidity.

Recent data showed that formerly preeclamptic women have a five to fourteen fold higher risk for developing end stage renal disease (ESRD).[4,5] Moreover, women who experienced multiple preeclamptic pregnancies have an even higher risk for ESRD.[4] The risk for developing cardiovascular disease (CVD) is especially high for women who have a history of early-onset preeclampsia (before 34 weeks of gestational age).[3] It is unknown whether this also applies for the risk of developing ESRD. However, in a large Norwegian cohort study the association between former PE and developing ESRD is stronger in formerly preeclamptic women who had given birth to preterm infant or child with low birth weight. Early-onset preeclamptic women often give birth to a preterm infant or child with low birth weight. Therefore, this suggests that these women might have a higher risk for developing ESRD.[4] The exact mechanisms underlying the increased risk for CVD and ESRD in formerly preeclamptic women are not completely understood.[6]

There are data, albeit sparse, showing that formerly preeclamptic women have persistent abnormalities in renal hemodynamics early and late after pregnancy, as a possible early pathway of increased renal risk.[7,8] However, this was mainly found in hypertensive women and thus might relate to the hypertension per se, rather than to the former PE specifically. Moreover, it is important to note that the renal hemodynamic profile is closely interlinked with sodium homeostasis and the renin-angiotensin aldosterone system (RAAS).[9] Sodium intake modulates renal hemodynamics in healthy subjects[10] as well as in subjects with hypertension.[11] In risk populations like sodium-sensitive hypertensive and overweight subjects, high dietary sodium intake elicits an unfavorable renal hemodynamic profile, which is absent during low sodium diet.[12,13] With regard to blood pressure response, both sodium sensitivity and altered response to angiotensin II (ang II) is reported in formerly preeclamptic women.[14-16] The role of sodium intake in renal hemodynamics and the renal response to ang II in relation to sodium intake in formerly preeclamptic women is still unknown.

Therefore, in the present study, we investigated the renal hemodynamic profile in women with a history of early-onset PE, compared with healthy controls during both low and high sodium intake. To address the effect of prior PE itself we carefully selected healthy normotensive Caucasian formerly preeclamptic women, without co-morbidity, with a body mass index (BMI) ‹ 30 kg/m² and excluded hypertensive formerly preeclamptic women. In steady state during low and high sodium intake, glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and filtration fraction (FF) were measured at baseline and during graded ang II infusion. We

hypothesized that prior PE would be associated with changes in the renal hemodynamic profile, be it or not dependent on sodium intake, as candidate mechanism for the increased risk for long-term renal damage in formerly preeclamptic women.

METHODS

Study population

We identified 264 formerly early-onset preeclamptic women (referred to as formerly preeclamptic women) one to ten years after delivery from an electronic delivery database of the department of Obstetrics and Gynecology at the University Medical Center Groningen (UMCG). Medical records were reviewed for accuracy of diagnosis of PE. PE was defined according to the International Society for the Study of Hypertension in Pregnancy criteria.[17] Early-onset PE was defined as developing PE before 34 weeks of gestational age. A total of 224 formerly early-onset preeclamptic women were invited by mail to participate in the study. Twenty-four of these women were willing to participate and were invited for a screening visit to the UMCG. After the screening visit, one women was excluded for using antihypertensive medication, one woman because of high blood pressure during the screening visit, one woman was using hormonal suppletion which could not temporarily stopped and three woman were excluded for other reasons (pregnancy, time consuming, post-menopausal). Each of the 18 remaining formerly preeclamptic women was matched for age and year of index pregnancy (within one year) with a parous control (referred to as control group) whose pregnancy had been uncomplicated and normotensive. These women from the control group were recruited either through the department's electronic delivery database or by advertisement. Their records were evaluated to confirm that pregnancy was indeed uneventful.

All women were non-smokers and normotensive, having a sitting systolic blood pressure ‹140 mmHg and diastolic blood pressure ‹90 mmHg measured at screening by an automatic sphygmomanometer (Dinamap®, G.E. Medical Systems, Milwaukee, Winsconsin, USA) and were not treated with an antihypertensive drug. Blood pressure was measured at screening at both arms to check for presence of a clinical significant difference in blood pressure (present in none of the subjects). Physical examination and electrocardiography did not reveal any abnormalities. None of the women had (a history of) underlying renal disease or hypertension, nor were they obese (i.e. BMI‹30 kg/m^2 at screening). They also did not have diabetes or a history of gestational diabetes, nor were they currently pregnant or lactating or using oral contraception. The study was approved by the local medical ethical committee (METc-number: 2010/294) and all women gave written informed consent in accordance with the Declaration of Helsinki. The study was registered in the Netherlands National Trial Register (www.trialregister.nl; trial registration number: 2635) as Response To Angiotensin II in formerly Preeclamptic women (RETAP) study.

Study protocol

This cross-over protocol consisted of two one-week periods with at least four weeks in between, a 7-day period on low sodium diet (LS; aim: 50 mmol Na^+/day) and a 7-day period on a high sodium diet (HS; aim:200 mmol Na^+/day). For assessment of dietary compliance and the achievement of a stable sodium balance 24-hour urine was collected at day 3 and day 6 during each period. During the last day of the dietary week, blood pressure was measured during a period of 24-hours by ambulatory blood pressure measurement (ABPM; Spacelabs Healthcare). The cuff was placed around the non-dominant arm at the brachial level. The recorders were programmed to measure blood pressure at a 20-min interval during daytime and at an hourly interval during nighttime (10pm till 6am). Women were asked to fill out a diary during this 24-hour to differentiate between day- and nighttime measurements and to correct for intense exercise afterwards. The nocturnal fall in blood pressure (dipping) was defined as the percentage decline in nocturnal blood pressure as compared to daytime values. In our study, non-dippers were defined as individuals with less than 10% decline in nocturnal blood pressure as compared to daytime blood pressure.

Both renal hemodynamics and ang II responsiveness are greatly influenced by sex hormones.[18] To avoid influence of these hormones, all measurements were performed during the mid-follicular phase (day 7±2 of menstrual cycle). At day 7 of both study periods, the subjects reported at the research unit at 8am after an overnight fast. Body weight, length and waist-to-hip ratio were measured at the start of this day. An intravenous cannula was inserted into each forearm, one for drawing blood samples, the other for infusion of radio-labeled tracers and ang II. Subjects received standardized meals and fluids during the day, with sodium intake adjusted to the prescribed diet. To ensure sufficient urine output, infusion of 250 mL/h of 5% glucose was administered and every hour 250 mL of oral fluids were provided.

GFR and ERPF were measured from the clearance of constantly infused radio-labeled tracers, ^{125}I-iothalamate (IOT) and ^{131}I-Hippuran (HIPP), respectively, in semi-supine position in a quiet room as described before.[19,20] After drawing a time point-0 blood sample, a priming solution containing 20 ml infusion solution (0.04 MBq of IOT and 0.03 MBq of HIPP) plus an extra amount of 0.6 MBq of IOT was given at 08.00 h, followed by a constant infusion of 12 ml/h. Plasma concentrations of both tracers are allowed to stabilize during 1.5-h equilibration, which is followed by two 2-h periods for simultaneous clearances of IOT and HIPP. The latter are calculated as $(U*V)/P_{IOT}$ and $(I*V)/P_{HIPP}$, respectively. U*V represents urinary excretion of the tracer; I*V, the infusion rate of the tracer, which equals clearance from plasma during steady state; P, tracer values in plasma at the end of each clearance period. The plasma clearance $(I*V)/P_{HIPP}$ equals its urinary clearance because there is no extrarenal clearance of this tracer. Thus, when plasma levels are in steady state, ERPF equals $I*V/P_{HIPP}$. GFR is calculated as the urinary clearance of IOT, corrected for voiding errors: $(U*V/P)_{corr.}$ As urinary clearance of HIPP equals plasma clearance in case of perfect urine collection, we routinely use the ratio of plasma-to-urinary clearance of HIPP to correct urinary clearance of IOT for voiding errors and dead space. By this method, coefficient of variation for GFR is 2.5% and for ERPF 5%. FF was calculated by the ratio of GFR and ERPF. Extra cellular volume (ECV) was estimated from the distribution volume of IOT and is calculated from the plasma level of IOT in the body,

which equals the amount infused minus the amount excreted. It is calculated as ((I*V+B*V)-(U*V))/P. B*V represent the bolus infusion of the tracers.[20] GFR, ERPF and ECV were indexed for body surface area (BSA), by dividing the raw sample by BSA and multiplying it with 1.73m². BSA was calculated according to the DuBois-DuBois formula.[21]

Blood pressure and heart rate were measured by using an automated sphygmomanometer (Dinamap; GE Medical Systems, Milwaukee, Wisconsin, USA) at 15-min intervals, with subjects being in a quiet room, in a semi-supine position, with their arm in resting position. Appropriate blood pressure cuff was determined on the basis of arm circumference. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the pulse pressure. Renal blood flow (RBF) was calculated as ERPF/(1–hematocrit). Renal vascular resistance (RVR) was calculated as MAP/RBF × 80,000. Baseline values for blood pressure and GFR and ERPF were obtained from 10am to 12pm. Between 12pm and 3pm ang II (Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) was administered intravenously, at a constant rate in doses of 0.3, 1 and 3 ng/kg/min each during 1-hour. During these ang II infusions blood pressure was measured at 5-min intervals.

Sampling and chemical analysis of urine and blood samples

Fasting blood samples were drawn for analysis of hematocrit (Ht), glucose, HbA1C, sodium, potassium, creatinine, and thyroid stimulating hormone (TSH). Measurements were performed by the use of an automated clinical chemistry analyzer (Sysmex hematology analyzer (for Ht), Sysmex Tosoh G8 (for HbA1c) and Roche Modular). Fasting serum insulin was determined by an automated immunoassay analyzer (Architect, Abbott). Homeostasis model assessment (HOMA) was calculated by: glucose (mL/L) x insulin (microunits/mL)/22.5. Blood for measuring plasma aldosterone and plasma renin activity (PRA) was collected at 11am in precooled tubes and immediately centrifuged at 4°C for 10min (3000 rpm). Plasma was subsequently stored in -80°C until analysis. Aldosterone was measured with a commercially available radioimmunoassay kit (coat a count RIA, Siemens). PRA was determined by a radioimmunoassay kit that detects the production of angiotensin I due to the enzymatic activity of plasma renin acting on endogenous plasma angiotensinogen (nanograms of angiotensin I produced per liter of plasma per hour; RIA, CisBio International, France). Urine samples were drawn from the 24h-urine collected by all women. The level of urinary sodium, potassium, creatinine, and albumin were assessed by the use of an automated clinical chemistry analyzer (Roche Modular Basel). As some study subjects were still slightly menstruating during the 24-hour urine collections, these samples were not suitable for albuminuria measurement. Therefore, to test for albuminuria, a random morning urine sample of all subjects was collected after the end of the study, at a point in time where subjects were certain not to menstruate to exclude confounding by admixture of blood.

Statistical analysis

Data were analyzed using SPSS 20.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA, USA). Parametric data are presented as mean ± standard deviation (SD) in text, tables and figures. Non-parametric data are expressed as median with 25th and 75th percentile. Differences in baseline characteristics, blood and urinary parameters between formerly preeclamptic women and controls were tested with the Student *t*-test for parametric data and Mann-Whitney U test for non-parametric data. For 24-hour blood pressure data, dipping was analyzed with the Chi-square test. For MAP and renal hemodynamics, to separately test the effects of sodium intake (factor diet), history of preeclampsia or not (factor group), and the interaction between the two (factor diet*group), a generalized estimating equations (GEE) analysis was performed. In the GEE analysis we corrected FF and MAP for difference in BMI. This repeated measurements analysis is appropriate for this small cross-over study with repeated measures in one subject.[22] In comparisons a p-value ‹0.05 was considered statistically significant.

Power calculation

The cross-over design of the study with multiple factors resulted in a multivariate power calculation. The main endpoint of the RETAP study was renal response (GFR, ERPF and FF) to ang II after low and high sodium diet in formerly preeclamptic subjects compared to healthy controls. In the multivariate power calculation 3 factors (response to ang II, low and high sodium diet and control group vs. formerly preeclamptic women) and 2 confounders were taken into account. Therefore, 25 women per group (n=10*5/2) were needed to perform multivariate analysis. Due to the low incidence of early-onset PE and the demanding nature of the study, we were not able to include 25 women per group in our hospital. However, after including 18 women per group and performing an interim analysis, we found a significant difference between both groups.

RESULTS

Baseline characteristics

Baseline characteristics of the two groups are shown in table 1. There were no statistically significant differences in age, number of pregnancies (gravidity), number of births (parity) and time since last pregnancy between the two groups. 15 out of 18 women experienced the early-onset preeclampsia during their first pregnancy, the other three women experienced preeclampsia during their second pregnancy. Formerly preeclamptic women had a higher weight and consequently a higher BMI compared with the control group. Both groups showed an increase of approximately 1.5 kilogram in weight during high sodium intake compared to low sodium intake (p‹0.001). Waist-to-hip ratio was not significantly different between the two groups.

The average 24-hour blood pressure values are shown in table 1 and were similar in formerly preeclamptic women and control subjects. Both groups responded

to high sodium intake reflected by an increased blood pressure. However, the average salt-induced increase in blood pressure did not differ between the two groups. Data are presented for 24-hour averages but similar results are present when analyzing diurnal and nocturnal values separately. The number of women showing the nocturnal fall of MAP (dipping pattern) was not significantly different between the two groups.

Table 1. Baseline characteristics

Characteristic	History of normotensive pregnancy (n = 18)	History of preeclamptic pregnancy (n = 18)	P
Age, years	36 ± 5	36 ± 5	.951
Gravidity	2.5 ± 1.3	2.6 ± 1.1	.951
Parity	2.0 ± 0.7	2.2 ± 1.0	.589
Time since last pregnancy, years	4.2 ± 2.6	5.3 ± 3.0	.243
Weight LS, kg	66.1 ± 8.3	73.2 ± 10.5	**.029**
Weight HS, kg	67.9 ± 8.3*	74.9 ± 11.0*	**.039**
BMI LS, kg/m^2	22.6 ± 2.6	25.3 ± 3.3	**.010**
BMI HS, kg/m^2	23.2 ± 2.7	25.9 ± 3.5	**.015**
Waist-to-hip ratio	0.83 ± 0.04	0.84 ± 0.06	.443
24-h MAP LS, mmHg	87 ± 5	88 ± 8	0.89
24-h MAP HS, mmHg	90 ± 7*	90 ± 8*	0.71
Dipping MAP LS no/yes (%yes)	1/17 (94 %)	3/13 (81%)	0.23
Dipping MAP HS no/yes (%yes)	3/15 (83%)	5/11 (69%)	0.32

*Data are presented as mean ± SD. BMI, body mass index; MAP, mean arterial blood pressure; LS, low sodium; HS, high sodium. *p<0.05 vs low sodium within the group.*

Blood and urinary parameters

Blood and urinary parameters are shown in table 2. No statistically significant differences in hematocrit, fasting glucose, insulin, HOMA, HbA1C, plasma sodium, plasma potassium, plasma creatinine, and TSH were found between the two groups. In both groups, plasma creatinine was significantly lower during high sodium intake compared with low sodium intake (p=0.001).

No statistically significant differences in urinary sodium excretion, potassium excretion and urea excretion were found between the groups; this reflects an equal intake of sodium, potassium and proteins between the two groups. Formerly preeclamptic women had a higher urinary creatinine excretion compared with control subjects.

No differences in PRA and aldosterone were found between the groups. In both groups, a significant decrease in PRA and aldosterone was found during high sodium intake compared to low sodium intake (p<0.001), which reflects that in both groups the systemic RAAS is adequately and similarly modulated by sodium intake. There were no differences in urinary albumin/creatinine ratio between the groups.

Table 2. Blood and urinary parameters

Parameter	History of normotensive pregnancy (n = 18)	History of preeclamptic pregnancy (n = 18)	P
Hematocrit LS, l/l	0.40 ± 0.02	0.40 ± 0.03	.460
Hematocrit HS, l/l	0.38 ± 0.03	0.38 ± 0.03	.634
Glucose LS, mmol/L	5.1 ± 0.7	5.0 ± 0.5	.628
Glucose HS, mmol/L	5.0 ± 0.5	5.0 ± 0.3	.605
Insulin LS, µU/mL	8.35 (5.60-9.90)	8.50 (6.50-12.80)	.542
Insulin HS, µU/mL	7.10 (4.70-9.30)	7.65 (4.60-10.80)	.525
HOMA LS	1.91 (1.26-2.16)	1.83 (1.33-3.01)	.636
HOMA HS	1.55 (0.97-2.15)	1.69 (1.08-2.30)	.369
HbA1c LS, mmol/mol	35 (31.75-36.00)	33 (32.50-35.00)	.134
HbA1c HS, mmol/mol	35 (32.75-37.25)	34 (30.75-35.25)	.203
Plasma sodium LS, mmol/L	140 ± 1.6	140 ± 1.9	.853
Plasma sodium HS, mmol/L	142 ± 1.8	141 ± 2.4	.164
Plasma potassium LS, mmol/L	4.0 ± 0.2	3.9 ± 0.3	.604
Plasma potassium HS, mmol/L	3.9 ± 0.2	3.9 ± 0.2	.287
Plasma creatinine LS, µmol/L	66.5 ± 9.2	70.1 ± 9.0	.242
Plasma creatinine HS, µmol/L	61.1 ± 7.0*	65.7 ± 9.3*	.090
Plasma TSH LS, mU/L	1.29 ± 0.6	1.60 ± 0.9	0.245
Plasma TSH HS, mU/L	1.48 ± 0.5	1.47 ± 0.9	0.975
Urinary sodium LS, mmol/24h	38.9 ± 14.0	45.1 ± 22.8	.326
Urinary sodium HS, mmol/24h	220.8 ± 63.5*	258.4 ± 85.9*	.145
Urinary potassium LS, mmol/24h	66.2 ± 21.3	76.3 ± 25.2	.202
Urinary potassium HS, mmol/24h	79.8 ± 33.5	73.3 ± 14.7	.459
Urinary creatinin LS, mmol/24h	9.8 ± 1.9	11.1 ± 1.0	**.013**
Urinary creatinin HS, mmol/24h	9.8 ± 1.9	11.5 ± 2.4	**.013**
Urinary urea LS, mmol/24h	264 ± 91	306 ± 63	.119
Urinary urea HS, mmol/24h	339 ± 89*	340 ± 65	.973
PRA LS, nmol Ang-I/L/h	0.80 (0.50-1.20)	0.85 (0.70-1.50)	.501
PRA HS, nmol Ang-I/L/h	0.20 (0.10-0.50)*	0.20 (0.09-0.30)*	.584
Aldosterone LS, pmol/L	255 (204-395)	341 (214-477)	.161
Aldosterone HS, pmol/L	71 (29-93)*	59 (35-96)*	.839
Urinary albumin/creatinine,g/mol	0.6 ± 0.3	0.5 ± 0.4	.212

*Data are expressed as mean ± SD or as median (25^{th}-75^{th} percentile). LS, low sodium; HS, high sodium; HOMA, homeostatic model assessment index; HOMA was calculated as [glucose*insulin/22.5]; PRA, plasma renin activity; TSH: thyroid stimulating hormone. *p<0.05 vs low sodium within the group.*

Blood pressure and renal function at baseline

Blood pressure and renal function during high and low sodium intake are shown in table 3 and table 4, and figure 1. By performing GEE analysis, we found no differences in MAP between both groups (p_{group}=0.401). High sodium intake significantly increased MAP in both groups to a similar extent (p_{diet}<0.001; $p_{diet*group}$=0.414, no interaction between diet and group).

With regards to renal hemodynamics, no differences were found in GFR (p_{group}=0.688) and ECV (p_{group}=0.973) between both groups. Formerly preeclamptic women have a slightly lower ERPF compared to control subjects, but this did not reach statistical significance (p_{group}=0.253). However, FF was significantly higher in formerly preeclamptic women compared to healthy controls (p_{group}=0.035). High sodium intake significantly increased GFR, FF and ECV in both groups to a similar extent (GFR: p_{diet}<0.001, $p_{diet*group}$=0.824; FF: p_{diet}=0.025, $p_{diet*group}$=0.460; ECV: p_{diet}<0.001, $p_{diet*group}$=0.766). However, there was no effect of sodium intake on ERPF (p_{diet}=0.127, $p_{diet*group}$=0.683). The higher FF in formerly preeclamptic women was not explained by MAP; no significant correlation could be detected between MAP and FF (r=0.095; p=0.581).

Table 3. Baseline renal function parameters

Parameter	History of normotensive pregnancy (n = 18)	History of preeclamptic pregnancy (n = 18)	P
RVR LS, dyne*s/cm^5	10985 ± 2936	11026 ± 2017	.962
RVR HS, dyne*s/cm^5	11248 ± 3071	11461 ± 2388	.819
ERBF LS, mL/min/1.73m^2	625 ± 146	622 ± 99	.949
ERBF HS, mL/min/1.73m^2	642 ± 149	621 ± 105	.623
Creatinine clearance LS, mL/min	107 ± 28	111 ± 17	.591
Creatinine clearance HS, mL/min	114 ± 24*	124 ± 25*	.220
eGFR LS, mL/min/1.73m^2	102 ± 14	97 ± 15	.368
eGFR HS, mL/min/1.73m^2	109 ± 10*	103 ± 15*	.117

*Data are presented as mean ± SD. LS, low sodium; HS, high sodium; RVR, renal vascular resistance; eGFR, estimated glomerular filtration rate. eGFR was calculated by using CKD-epi formula [36]. *p<0.05 vs low sodium within the group.*

Blood pressure and renal function during ang II infusion

Graded ang II infusion showed in both groups a dose-dependent rise in blood pressure during both high and low sodium intake (table 4). No significant differences were found in absolute blood pressure values during ang II infusion between both groups. Figure 2 demonstrates ERPF during ang II infusion. Both groups showed a dose-dependent decrease in ERPF during ang II infusion (p_{dose}<0.001). No differences were found in the responses of ERPF to ang II between the groups (p_{group}=0.337). Sodium intake did not affect the response of ERPF to ang II infusion (p_{diet}=0.562).

Table 4. Blood pressure at baseline and during angiotensin II infusion

MAP during ang II infusion	History of normtensive pregnancy (n=18)	History of preeclamptic pregnancy (n=18)	P
Low sodium			
Baseline, mmHg	81 ± 7	83 ± 8	.375
0.3 ng/kg/min, mmHg	81 ± 8	82 ± 8	.745
1.0 ng/kg/min, mmHg	86 ± 9	87 ± 9	.705
3.0 ng/kg/min, mmHg	92 ± 12	95 ± 10	.477
Recovery, mmHg	85 ± 8	87 ± 10	.612
High sodium			
Baseline, mmHg	85 ± 8	86 ± 9	.714
0.3 ng/kg/min, mmHg	84 ± 9	85 ± 10	.601
1.0 ng/kg/min, mmHg	89 ± 9	93 ± 11	.260
3.0 ng/kg/min, mmHg	99 ± 8	100 ± 10	.646
Recovery, mmHg	89 ± 8	89 ± 11	.981

Data are presented as mean ± SD. MAP, mean arterial pressure; ang II, angiotensin II.

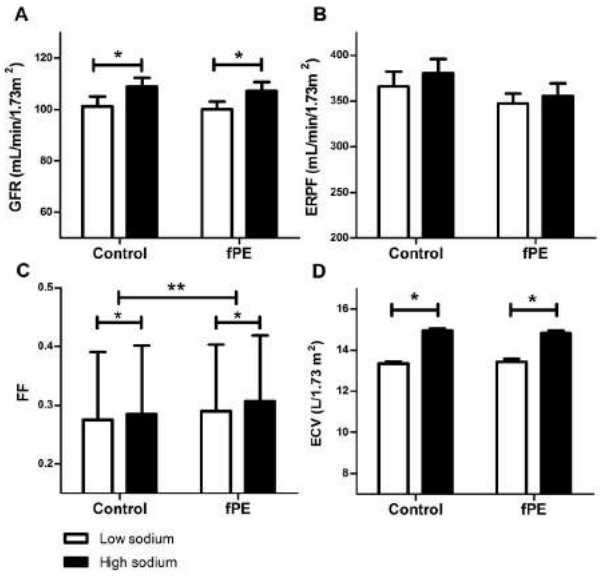

***Figure 1**: Glomerular filtration rate (GFR) **(A)**, effective renal plasma flow (ERPF) **(B)**, filtration fraction (FF) **(C)** and extra cellular volume (ECV) **(D)** at baseline during low sodium (white bars) and high sodium (black bars) diet in women with history of normotensive pregnancy (control) and in formerly preeclamptic women (fPE). Data are expressed as mean ± SEM * p<0.05 low vs high sodium intake, **p<0.05 control vs fPE (GEE analysis; FF is corrected for BMI). No interaction between dietary sodium response and group.*

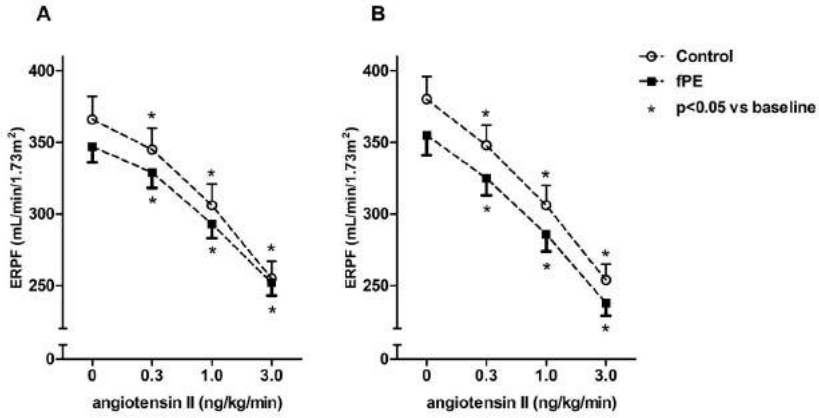

***Figure 2**: Effective renal plasma flow (ERPF) during angiotensin II infusion during low sodium **(A)** and high sodium **(B)** in formerly preeclamptic women (fPE) and women with history of normotensive pregnancy (control). No significant differences were found between the groups (GEE-analysis).*

DISCUSSION

This is the first study investigating renal hemodynamics in healthy formerly early-onset preeclamptic women compared with women with a history of a normotensive pregnancy, during a low and high sodium diet with graded ang II infusion. Although blood pressure was not different, a slightly, but significantly higher FF was present in formerly early-onset preeclamptic women on either sodium intake. There was no difference in renal response to ang II infusion during either high or low sodium intake. Thus, healthy women with a history of early-onset PE, but without any co-morbidity, have slight but persistent subtle differences in renal hemodynamics compared to matched controls, irrespective sodium intake. The important question whether formerly preeclamptic women exhibited impairments in renal function pre-pregnancy, and thereby affecting our results, remains unanswered. Prospective long-term follow-up studies investigating renal hemodynamics (i.e. by the means of eGFR) in formerly preeclamptic women and healthy controls are warranted to gain more insight whether our subtle differences in renal hemodynamics, increased FF, lead to renal dysfunction in the long-term in formerly preeclamptic women.

Our study is the first to report renal hemodynamic effects of prior PE in a population of women without co-morbidity. Prior studies in formerly PE women described more pronounced changes in renal hemodynamic profile, but in these studies the co-morbidity, namely hypertension, might well explain the renal hemodynamic findings of a higher FF and RVR with lower ERPF.[7,8] 24-hour blood pressure measurements and blood pressure response to sodium intake were all comparable between our two study groups reinforcing that our study population was indeed very healthy. A recently published study in women 5-10 years after severe early-onset PE showed marked

increased sodium sensitivity.[23] In line, in three previous studies reporting increased responsiveness to ang II of blood pressure in the postpartum period[14,15,24] the patient selection, including a mix of phenotypes (gestational hypertension and late- or early-onset PE, and lack of exclusion of co-morbidity), did not allow to study the effect of prior PE per se.

The assumption that PE per se may predispose to altered renal function and kidney damage is in line with other findings. Chambers et al. demonstrated impaired endothelial function in women with a history of PE, independent of established risk factors.[25] Furthermore, in a large epidemiological study, Vikse et al. reported that familial aggregation of risk factors does not explain the increased ESRD risk after PE.[26] Animal studies could elucidate whether ESRD after PE is induced by PE or by other factors, but data on mother's vascular outcome are sparse. On the contrary of the hypothesis that PE itself might lead to an impaired renal hemodynamic profile post-partum, a particular renal hemodynamic profile pre-pregnancy increasing the risk for PE during pregnancy and influencing long-term renal health is also a plausible hypothesis. In for example an experimental sFlt-1 mice modelblood pressure and vascular reactivity was not different 6-8 months after delivery.[27]

Microalbuminuria has been thought to be present after PE, as described in a meta-analysis with a mixed patient population (including diabetes mellitus).[28] However, in a recent large Norwegian study, PE was not associated with increased risk of persisting microalbuminuria.[29] In line with the results from Sandvik et al, a morning urine sample collected after completion of the study did not show a difference in albuminuria between the groups; all women had albuminuria values within the normal range.

A higher FF, even within the normal range, can be considered a candidate mechanisms for development of hypertension and renal damage, as proposed by Brenner et al.,[30] mainly based on micropuncture studies in rats. Based on these, an increased FF is assumed to be a proxy for elevated glomerular capillary pressure, thus contributing to progressive renal damage during long-term exposure.[31] Data on the pathogenetic role of elevated FF in human are scarce, but our own group has previously shown that a mild elevation of FF is associated with worse long term renal outcome and mortality in renal transplant recipients independent of all other risk factors.[32]

The mechanism underlying the higher FF in current study cannot be established with certainty, as this would require micropuncture. Hemodynamic as well as structural differences of the glomerular microvasculature should be considered. The nominally lower ERPF in the formerly preeclamptic women is compatible with a shift in glomerular vasotonus towards more efferent vasoconstriction relative to afferent vasotonus. Several neurohumoral factors could elicit such a pattern, alone or in combination, including increased activity of the RAAS and the sympathetic nervous system, vasopressin, natriuretic peptides and/or other factors.[31] We found no differences, however, in circulating parameters of RAAS-activity or in ang II renal responsiveness. Whereas differences in tissue RAAS-activity could be involved, the possibility is not supported by similarity in renal ang II response. Reduction of increased intraglomerular pressure by the use of RAAS blockade in healthy subjects underlies the role of the RAAS in impaired renal hemodynamics, i.e. hyperfiltration.[33] Also the progression to chronic kidney diseases is delayed by RAAS blockade, potentially

through influencing the mechanical forces on the filtration barrier by affecting the filtration rate.[34] A difference in filtration equilibrium could also be involved, but this cannot be assessed directly in human. Differences in sodium and protein intake are not likely to be causative for the increased FF found since urinary sodium and urea levels were comparable between the groups indicating an equal intake of both. Finally, structural differences in the glomerular microvascular bed could be involved, although the reduction in FF during sodium restriction demonstrates that there is at least a partial hemodynamic component. So far, it is thought that glomerular changes (glomerular endotheliosis, accompanied by decreased GFR) during preeclampsia resolve completely after preeclampsia.[35]

High sodium intake induced an increase in FF in both groups. This effect of high sodium intake on renal hemodynamics is in line with studies in sodium-sensitive hypertensive individuals and overweight subjects, which demonstrated hyperfiltration elicited by high sodium intake.[12,13] Since we had a small difference in BMI between both groups, we corrected in our multi-analysis for BMI. Independent of BMI we found a difference in FF between both groups. Furthermore, we did not find an interaction between the effect of sodium intake on FF and prior PE. However, considering the effect of high sodium on renal hemodynamics and the aligned role of increased FF in the risk for long-term renal damage, our data suggest that sodium restriction could exert a beneficial effect on long-term renal risk. Obviously long-term studies would be required to substantiate such an assumption. The possible beneficial effect of sodium restriction is supported by Martilotti et al. showing sodium-sensitivity of blood pressure in formerly preeclamptic women.[23] However, Martillotti et al. included women with comorbidity (i.e. increased blood pressure and microalbuminuria) so future studies in a well-defined population should confirm sodium sensitivity in order to start the optimal preventive prophylactic interventions including life-style modification in these women with a high cardiovascular risk profile.

Our study has several limitations. Firstly, due to our strict inclusion and exclusion criteria our sample size is relatively small, and inclusion was ended before the number of women calculated using power calculation were included. In addition, the range in years post-partum is relatively broad although not different between the two groups. Furthermore, our study lacks mechanistically data (i.e. sympathetic nervous system assessment) and diet was not standardized for protein intake. At last, the question whether it is PE itself or pre-pregnancy renal function impairments leading to the post-partum increased FF in formerly preeclamptic women remains unanswered.

In conclusion, formerly early-onset preeclamptic women, in the absence of co-morbidity, show an altered renal hemodynamic profile characterized by a slightly, but significantly higher FF as compared to healthy matched controls. Taken into account the absence of co-morbidity, these data fit the assumption that altered renal hemodynamics are at least partly induced by PE itself. Future studies are warranted to evaluate whether these altered renal hemodynamics independently of co-morbidity, contribute to the increased risk for ESRD on the long-term in formerly preeclamptic women.

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